

**Effects of Induced Rumen Acidosis on the Fecal Shedding of *Escherichia coli* in Lactating
Dairy Cattle**

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Abstract

Rumen acidosis was induced in late lactation dairy cows to measure the effects in manure microbial output. Rumen acidosis was induced in 5, late-lactation Holstein cows by a diet moderately high in starch. Five other cows were fed a control diet. Fecal samples were collected five times during the 10 d trial to enumerate Gram-negative bacteria, coliform, and *Klebsiella* species in manure. The pH also was measured in each fecal sample. Diets were assayed for nutritional composition throughout the trial. Rumen acidosis, as confirmed by milk fatty acid profiles, did not increase the fecal shedding of *E. coli* or other organisms enumerated. Although no treatment effects on the fecal bacteria counts were measured, daily differences in fecal bacteria counts and pH were observed. Milk production increased 2.2 kg/d in cows fed the acidosis inducing diet compared to cows fed the control diet. Cows fed the acidosis inducing diet had increased milk concentrations of the fatty acid *trans*-10, *cis*-12 conjugated linoleic acid compared with milk from control cows. The effect of diet on fecal shedding of *E. coli* was not different by treatment. Nutritional changes in the dairy herd can affect pathogen exposure; however, this was not observed.

Introduction

Intensive management of food animals in confinement housing systems has the potential for increasing the frequency of infectious disease due to increased pathogen exposure. In dairy cattle, rumen acidosis is a major contributor to early lactation metabolic disorders, but it also can lead to other health problems, including mastitis, metritis, pneumonia, dystocia, reproductive incapability, decreased production, and many other sequelae (Enemark et al., 2002). Mastitis, or inflammation of the mammary gland, is a major economic issue in the dairy industry, costing on average \$200/cow/year. The actual costs of mastitis were determined by treatment supplies, veterinary fees, labor costs, loss of milk production, and replacement costs (Smith and Hogan, 2002). Mastitis is often caused by an intramammary infection (IMI) from contagious or environmental pathogens. Current research has shown relationships between diet contents and fecal shedding of opportunistic pathogens. Callaway et al. (2003) discussed the need for research on limiting exposure to enterohaemorrhagic *Escherichia coli* by decreasing the available feedstuffs in the colon. Increased rumen acidity leads to an increase in endotoxin in the rumen digesta and blood, but it is not etiologically made by the lysis of gram-negative bacteria in the rumen. Gram-negative bacteria actually increase in number in the gastrointestinal tract during acidosis of the rumen (Nagaraja et al., 1978).

Increased pathogen load in the cow's environment is a potential source of IMI and is directly related to the animal's health and welfare. Understanding how to control teat end exposure to IMI-causing pathogenic organisms may prove an economic interest in the scope of the producer's financial asset in the animal and the cost of production. Reducing the available pathogens in the cow's environment may also prove a successful attempt at limiting the occurrence of mastitis in dairy herds and provide a better well-being for dairy animals. The

objective of this study was to determine if an increase dietary concentration of starch would lead to an increase in fecal shedding of *E. coli*.

Materials and Methods

Ten late lactation Holstein cows were randomly selected from the research herd at the Ohio Agricultural Research and Development Center's Krauss Dairy Center. Six second-lactation cows and four first-lactation cows were paired by parity into control and experimental groups. Cows were housed in tie-stalls bedded with kiln dried sawdust. The control diet was consistent with the late lactation diet of the herd at that time of the year (Table 1). All cows were held on the control diet for a preliminary period of five days. The experimental period for acidosis fed cows was 3d more of control diet and then 7d on the acidosis diet (10d total experimental period). Cows in the experimental group diet changed from control to a high grain diet on d 3 of the experimental period. Control fed cows remained on the control diet during all 10d of the trial after the 5d preliminary period.

Fecal sampling, pH, and bacteriology

Fecal sampling occurred on d 1, 3 to 5, and 8 and 9 of the experimental period. Fecal samples were collected at 0800h on the specified sample days via direct collection from the rectum using long palpation sleeves. Approximately 250 g of manure was collected from each cow and held in the inverted sleeve for transport to the laboratory. Bacteriology was based on methods used in Hogan et al. (1999). On immediate arrival at the laboratory, within 30 min of collection, fecal pH was measured by mixing 10 g of manure with 90 mL of distilled water in 236 mL glass jars. For bacteriology, 5 g of feces were mixed into 45 mL of sterile phosphate buffered saline (PBS) in a 45 mL plastic centrifuge tube, creating a 1:10 dilution. A dilution series was then created using a tissue culture well plate up to a 10^{-5} dilution. The serial dilutions

(10^{-1} through 10^{-5}) were then spot plated on the surface of MacConkey agar (Becton, Dickson, and Co., Sparks, MD) and MacConkey-Inositol-Carbenicillin (MCIC) agar. Myo-inositol (5 g/L; Sigma-Aldrich, St. Louis, MO) and carbenicillin (75 μ g/mL; Pfizer, New York, NY) were added to MacConkey agar base (Becton, Dickson, and Co.) to make MCIC agar. Inoculated media was then incubated at 37°C for 24 h. Colony-forming units per gram were identified as Gram-negative bacteria (total growth on MacConkey agar), coliforms (lactose positive colonies on MacConkey agar), and *Klebsiella* spp. (pink to red colonies on MCIC). Bacterial counts were normalized by \log_{10} /g transformation.

Feeding and Diet Analysis

Diets consisted of corn silage, alfalfa silage, corn milling product, and a grain mix with added vitamins and minerals (Table 1). Cows were fed individually in an *ad libitum* fashion. Feed samples of each ingredient were taken three times during the trial and composited within treatment for laboratory analyses. Analyses on each ingredient included: dry matter (DM), ash, neutral detergent fiber (NDF), nitrogen, and starch. Milk samples were collected on d 9 to use for milk fatty acid analysis.

Statistical Analyses

Bacterial counts, pH, milk production, and milk fatty acid profiles were analyzed using ANOVA (SAS; Cary, N.C.). Milk fatty acids were analyzed by treatment and by lactation. Bacterial and pH data were compared for effects of treatment, day, and treatment by day interactions. Production data were analyzed from d 3 to the end of trial, as diets changed on d 3 from preliminary feeding.

Results

Bacterial Counts and pH Measurements

Dietary treatment had no effect on Gram-negative, coliform, or *Klebsiella* spp. bacterial counts from manure samples (Figures 1-4; $P > 0.05$). Gram-negative bacterial and coliform counts were affected by day, $P = 0.05$ and $P = 0.07$ respectively. Daily differences were consistent between treatment groups for both Gram-negative and coliform counts (Figures 1 & 2). Gram-negative bacterial and coliforms counts were greater ($P < 0.07$) on d 8 and d 9 than on d 5. *Klebsiella* species counts for both treatments were below detection level using the enumeration method described (Figure 3). Fecal pH was not affected by dietary treatment (Figure 4). Fecal pH did vary greatly by day ($P < 0.0001$). Differences in pH by day occurred between d 3 and 8 ($P = 0.01$) and d 3 and 9 ($P = 0.001$). More differences in pH by day occurred between d 4 and 8 ($P = 0.0002$); d 4 and 9 ($P = 0.01$); d 5 and 8 ($P < 0.0001$); d 5 and 9 ($P = 0.004$); and d 8 and 9 ($P < 0.0001$). Correlation between pH and bacterial counts of Gram-negative, coliform, and *Klebsiella* species were not observed ($P > 0.10$).

Milk Production and Diet

Daily DMI did not differ between treatment groups (Table 2; $P > 0.10$). After adjustment for initial milk production, a difference in milk yield between dietary groups was significant ($P < 0.05$). Cows on the experimental acidosis diet produced approximately 2.2 kg/d more milk. Yields of milk fat and protein did not differ between treatment groups ($P > 0.05$). Neither milk fat nor protein percentages were affected by dietary treatment ($P > 0.10$). Milk urea nitrogen (MUN) did not differ between treatment groups ($P > 0.10$); however, MUN for the acidosis treatment was 2 units higher for the control.

Milk Fatty Acid Analysis

Milk fatty acid profiles differed little between treatments. Of the fatty acids analyzed, concentration of *trans*-10, *cis*-12 conjugated linoleic acid was significantly higher in the milk

from acidosis treatment cows than control fed cows ($P < 0.05$). All other fatty acids analyzed [total *trans* fatty acids; *trans*-6, *trans*-8, 18-1; *trans*-9, 18-1; *trans*-10, 18-1; *trans*-11, 18-1; and *trans*-12, 18-1] were not significantly different between treatment groups ($P > 0.05$). No other fatty acids were analyzed.

Discussion

The effects of induced rumen acidosis on fecal shedding of *E. coli* did not differ between acidosis and control fed groups. No difference was noted between treatment groups based on the diets fed. Bacterial counts were similar to those from previous work. Previous research has shown increased, decreased, and no change in coliform counts based on diet changes in fiber and starch (Callaway et al., 2003). Coliform enumeration was considered to be *E. coli* by lactose-positive colonies on MacConkey agar. Coliform counts assayed in the current trial were not different between treatment groups, while previous research has shown increased coliforms based on increased starch concentrations. Bacterial counts did not differ between treatment groups, and this effect is possibly due to the minute nutritional differences in rations for acidosis and control groups. A noted effect by day was on d 5 when bacterial counts differed from those of other days. This trial was not as abrupt in concentration changes of starch and protein from control to the acidosis diet as observed in previous research (Callaway et al., 2003; Edrington et al., 2006; and Enemark et al., 2002) and may relate to the limited effects on bacterial (*E. coli*) populations. The increase in starch concentration of the diet may not have impacted the large intestine and colonic microbial community as previously noted, but did result in an increase in milk production. *Klebsiella* species counts were not detectable using the described method and were not expected to be significant. *Klebsiella* species counts in fecal material are highly

variable due to the intermittent infection and shedding of the organism from the digestive tract (Munoz and Zadoks, 2007; Podschun and Ullman, 1998).

Fecal pH was variable and did not correlate with a treatment effect. Fecal pH did differ day by day; however, fecal pH monitoring is highly dependent on time between sampling and measuring, and also management factors, such as barn cleaning, cattle movement, and feeding times. Fecal pH was carried out at 0850h on sampling days; approximately 4 h after morning feeding and 0.5 h after fecal collection.

Milk production was affected by treatment diet. Cows fed the acidosis diet had a higher average milk yield (25.4 kg/d) than those of the control group (23.3 kg/d). More starch, energy, and protein from the increased grain content were readily available for fermentation and utilization by the body for milk synthesis, which would lead to an increase in milk yield. Significant differences in milk fat and protein percentages may have been observed if a larger population of cows was used for trial. Given that only ten cows were used, significant differences in milk fat, protein, and MUN were not expected. While MUN for the acidosis group was higher (16.0 mg/dL) than that of the control (14.4 mg/dL), crude protein percentage in the acidosis diet was higher (17.6%) than for the control (16.6%) and thus had considerably more protein available.

Milk fatty acid profiles varied little between treatments, but they were indicative of an acidotic effect in the treatment group. A difference in the level of *trans*-10, *cis*-12 conjugated linoleic acid was noted. Acidosis cows had a higher concentration of this fatty acid, which can increase during rumen acidosis (Enjalbert et al., 2008). Cows were not clinical acidosis presenters; however, the increased *trans*-10, *cis*-12 conjugated linoleic acid concentration is indicative of a disruption in the normal biohydrogenation of fats in the rumen (Bauman and

Griinari, 2001). Milk fatty acid profiles generally fluctuate during acidosis due to the effects of acidosis on biohydrogenation of saturated fats in the rumen (Colman et al., 2010). Due to the limitations of one milk sample seven days after the start of the acidosis diet, fatty acid profiles were not monitored for changes over time and compared with pre-trial conditions.

Conclusion

Minor changes in starch and protein concentrations in dairy cattle diets may not have significant effects on fecal shedding of *E. coli*. Feeding and ration changes affect body systems and have a pronounced effect on fecal shedding of gastrointestinal tract bacteria. A future project may use a larger number of cows to review the small insignificancies from the current trial. Pathogen load exposure fluctuations in the modern animal agricultural housing systems can greatly increase chances of disease and economic loss, while affecting animal physiology and production.

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Table 1. Acidosis and control diet compositions

Dietary Component ¹	Control Diet	Acidosis Diet
Alfalfa Silage	33	20
Corn Silage	25	25
Corn Milling Product ²	22	17
Grain Mix	20	38
Dry Matter %	58.4	65.0
Organic Matter	92.8	93.1
Crude Protein	16.6	17.7
Neutral Detergent Fiber (NDF)	33.3	28.9
Forage NDF	23.4	18.1
Starch	24.5	30.2

¹ Components are listed as a percentage of dry matter, except where noted.

² “Dairy Protein Product,” Cargill, Minneapolis, MN

Table 2. Feed intake and milk production of acidosis and control groups

Cow	Lactation	Diet Fed	Body Wt. (kg)	Pair	DIM ¹	Milk (kg/d)	DMI ² (kg/d)
4844	2	Acid	755	1	296	23.9	20.7
4855	2	Control	795	1	256	24.9	18.4
4858	2	Acid	750	2	289	24.2	20.8
4869	2	Control	680	2	245	23.8	18.7
4874	2	Acid	670	3	247	24.0	16.1
4877	2	Control	695	3	253	27.8	21.9
4929	1	Acid	640	4	259	24.1	18.6
4940	1	Control	605	4	252	25.8	17.5
4942	1	Acid	592	5	249	29.4	21.2
4943	1	Control	620	5	230	30.3	18.4

¹ Days in milk

² Dry matter intake average to 10d trial

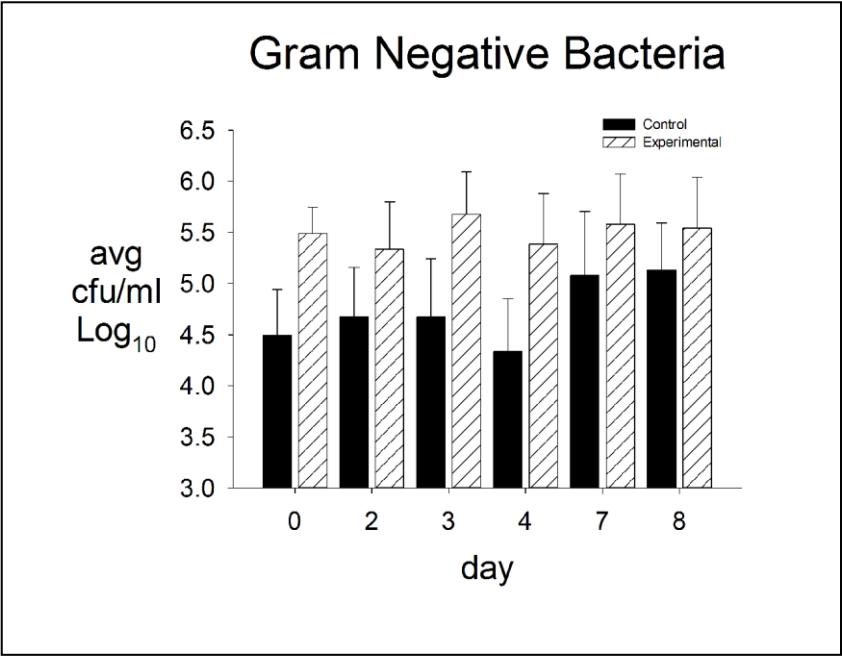


Figure 1. Gram negative bacteria count by day

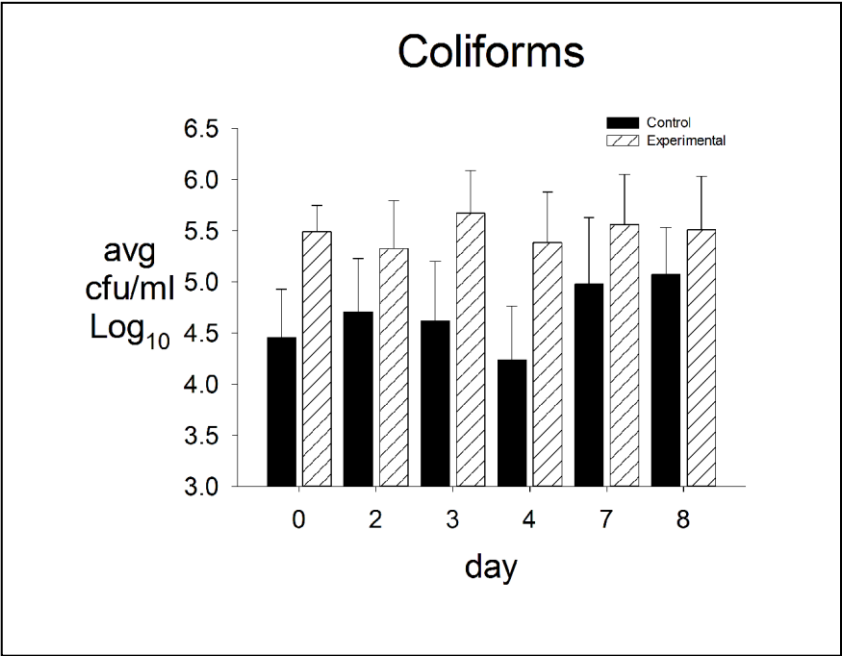


Figure 2. Coliform count by day

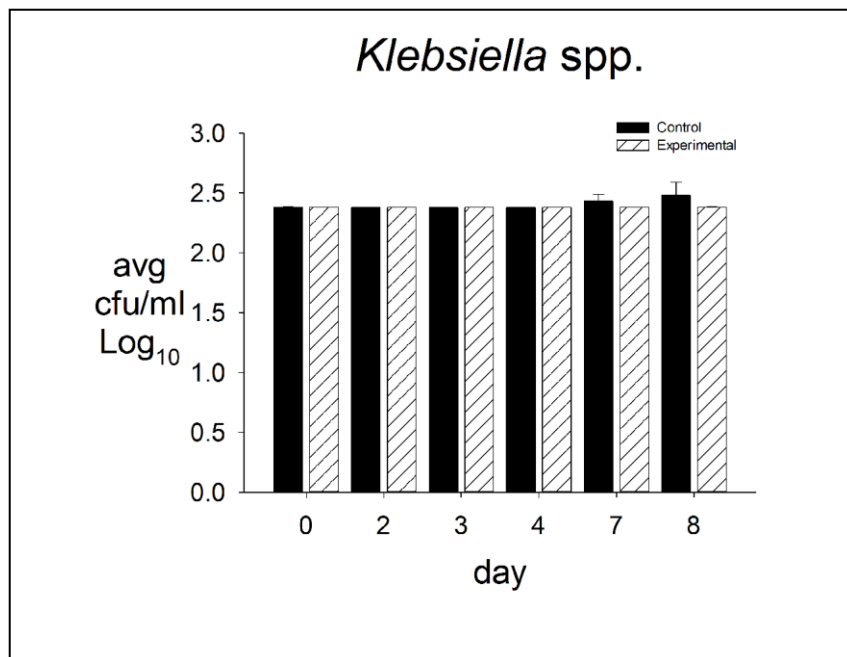


Figure 3. *Klebsiella* spp. count by day

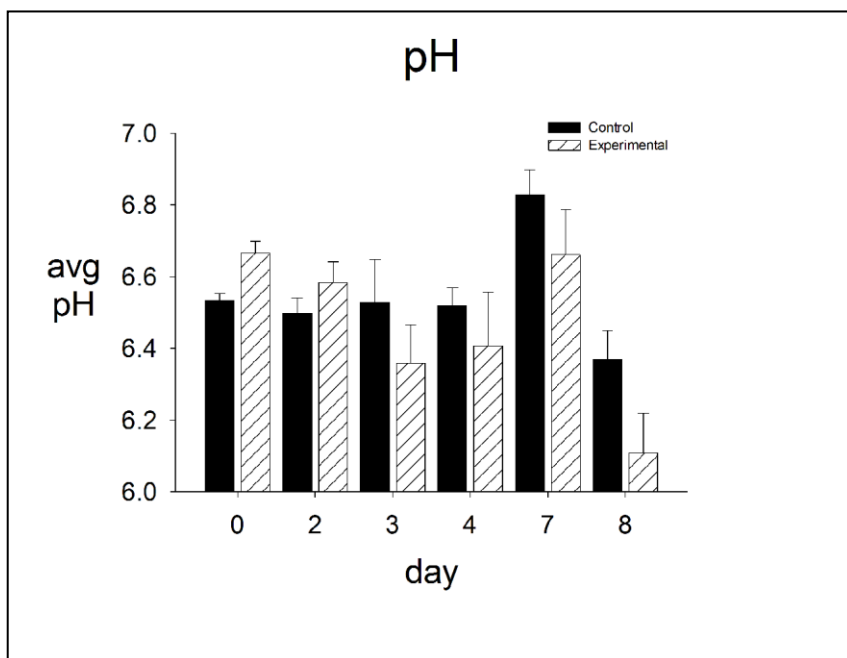


Figure 4. pH measurements by day